mals at the time of sacrifice. In this connection, the experiments of Lippman(10) have indicated that "drawn blood" values are a reflection of the actual blood volume levels. Also in line with the concept of a plasma volume change is the finding that a significant rise occurs in red cell counts and hemoglobin values within a few days following cessation of STH treatment in the hypophysectomized rat(6).

Summary. Administration of STH to hypophysectomized rats results in peripheral reticulocytosis and increases in the percentages of nucleated erythroid elements within the bone marrow. Considerable repair of the hypoplastic marrow is induced by this agent. A rise in peripheral erythrocytic values fails to occur probably because of a concomitant increase in plasma volume.

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Infrared Spectroscopy of Liver Glycogen in Normal and Irradiated Adult and Fetal Rats.* (20797)

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Previous studies(1) on rabbits demonstrated infrared absorption spectra of liver for the region 1-15 μ. Two types of absorption bands were found within 2 different intervals of that region: 1. Bands below 8.1 μ, which were very similar to those of the common "protein polyamide" vibrations(2) and occur not only in liver but in all other tissues (3.04 μ NH stretching, 3.4 μ CH stretching, 6.0 μ CO stretching, the deformation vibration of NH at 6.44 μ, the deformation vibration of CH at 6.84 μ, the CH 3 vibration at 7.26 μ, and an unassigned band at 8.1 μ). 2. Bands between 8.1 μ and 11 μ which have been shown to be characteristic for liver tissue, "fingerprint bands" (double bands at 9.70-9.64 μ and 9.23 μ). It was also noted that after 2-3 days starvation followed by insulin shock, one of the characteristic double bands at 9.70-9.64 μ disappeared, while the other at 9.23 μ remained, although greatly reduced in strength.

The present paper reports differences in infrared spectroscopic findings in the liver of adult and fetal rats, definite assignment of one characteristic band to glycogen, and spectroscopic studies of the effect of ionizing irradiation on liver glycogen in adult and fetal rats.

Materials and methods. Female albino rats of about 200 g were used for all experiments. Fetal livers were obtained in the 2nd-3rd week of development. Animals were kept for about one week on a balanced diet *ad libitum*, and then examined either non-fasted, fasted for 24 hours, or after feeding various sugars by stomach tube 4 hours preceding the ex-

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periment. When tube feeding was employed the tube was passed under light nembutal anesthesia on both experimental and control animals. All experiments were timed to take place at approximately 1 P.M. The animals were killed by decapitation and the livers quickly removed and frozen. For spectroscopy, frozen sections of 25-50 μ thickness were prepared, placed upon silver chloride plates and dried at low temperature. Histologic studies ruled out the possibility of complicating disease. The *irradiation* experiments comprised administering a single dose of total body irradiation of 600-19000 r to non-pregnant animals and of 150-400 r to pregnant animals. The technic of x-ray irradiation has been described elsewhere(3). Adult non-pregnant animals were sacrificed one or 2 days after the irradiation, fetuses 24 hours after maternal irradiation. The method of *infrared spectroscopy* of frozen and dried tissue sections has been described(4). For semi-quantitative estimate of the substances producing the characteristic band of liver tissue, ratios of optical densities of this band and a "protein vibration," *e.g.*, the deformation vibration of NH at 6.44 μ, were determined. Since such ratios between optical densities of unknown to internal standard are independent of thickness of sections according to Beer's law, they can be used for estimations in sections of varying thicknesses.

Results of qualitative infrared spectroscopy of adult and fetal liver tissue. The upper part of Fig. 1 gives transmittancy curves which were obtained from sections of about the same thickness (50 μ) of adult and fetal liver from non-fasted rats and from films of commercial glycogen.

The spectra of both adult and fetal liver show 2 different types of absorption bands similar to those found in rabbits, *e.g.*, "protein polyamide" vibrations below 8.1 μ and strong characteristic double bands at 9.70 μ-9.60 μ, and 9.23 μ, and weaker bands at 8.65 μ. Still weaker bands at 10.30 μ and 10.70 μ were seen in most of the spectral recordings, but do not appear regularly in the illustrations. Close inspection of the transmittancy curves indicates distinct differences between spectra of adult and fetal liver. The most

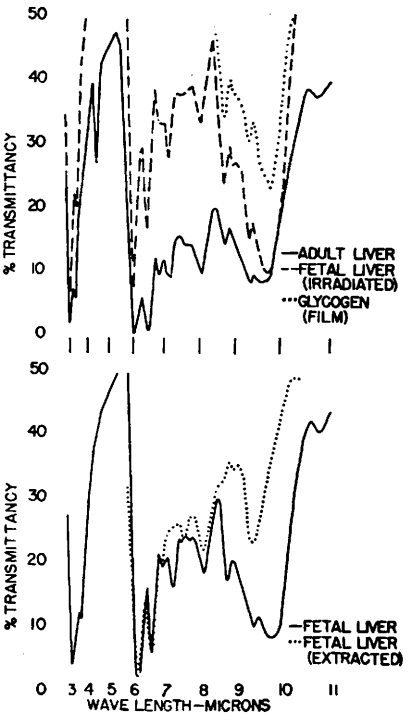

FIG. 1. Comparison of infrared absorption spectra of adult and fetal liver tissue and commercial glycogen (upper curves); effect of extraction with cold 10% trichloracetic acid on spectra of fetal liver (lower curves).

characteristic band of the tissue spectrum is located at somewhat lower wavelength in adult liver (9.60 μ) than in fetal liver (9.70 μ). The relative strength of those "specific bands" is always weaker than the strength of the "protein bands" at 6.44 μ in adult liver while it is almost equal or even stronger in fetal liver.

Assignment of characteristic bands of the liver spectrum. Glycogen is known to be contained in liver tissue in so high concentration that it seemed reasonable to investigate its role in producing the characteristic liver spectra. Comparison of the liver spectrum above 8.1 μ with the corresponding spectrum of commercial glycogen (upper part of Fig. 1) shows the great similarity of both spectra. Spectra of fetal liver and glycogen show identically located bands at 8.65 μ, 9.23 μ, and 9.70 μ. Spectra of adult liver show similar locations of bands with the exception of the characteristic double band which is found at somewhat lower wavelength (9.60 μ).

Extraction of mounted dried liver sections with either cold or hot chloroform, or cold or hot ethanol did not significantly affect the characteristic bands thus precluding any important contributions to the spectrum by lipids. Extraction of liver sections with cold 10% trichloracetic acid, on the other hand altered the spectra decisively. One of the characteristic double bands (9.70 μ-9.60 μ) was either completely absent or greatly reduced and the other at 9.23 μ although present, was much weaker after the extraction. The lower part of Fig. 1 demonstrates this effect of the extraction on a fetal liver section. The illustration, in addition, shows that the extraction, which so greatly changes the characteristic bands of liver tissue above 8.1 μ, does not affect the strength of the protein bands below 8.1 μ. Acid soluble phosphates which have weak absorption bands in the 9-10 μ region(5) are contained in liver in too small concentration to make any detectable contribution to the spectrum. It is, therefore, reasonable to assume that the trichloracetic soluble substance is glycogen, since only glycogen appears to have all 3 necessary qualities, *e.g.*, identical infrared spectra, sufficient tissue concentration, and solubility in cold trichloracetic acid. Additional proof for the assignment of the described infrared bands to glycogen has been obtained by comparing the liver spectra of adult fasted rats with liver spectra of animals given sugar after fasting and further comparsion of such spectra with glycogen (Fig. 2).

The liver spectrum of the fasted rat as a whole resembles that of liver extracted with cold trichloracetic acid, which has been considered to be practically glycogen-free. The liver spectrum of the sugar-fed rat, on the other hand showed the characteristic glycogen bands previously described except for slight variations in location of the strong specific band which was found at about 9.65 μ after sucrose and at 9.61 μ after glucose feeding. Contribution of glycogen to the liver spectrum of sugar-fed rats is even more suggested from difference curves which have been obtained by deduction of the absorption of "fasted liver" from the liver spectrum of the sugar-fed animals. The characteristic 8.1-11 μ region of such a difference curve (interrupted line) closely resembles a transmittancy curve of glycogen.

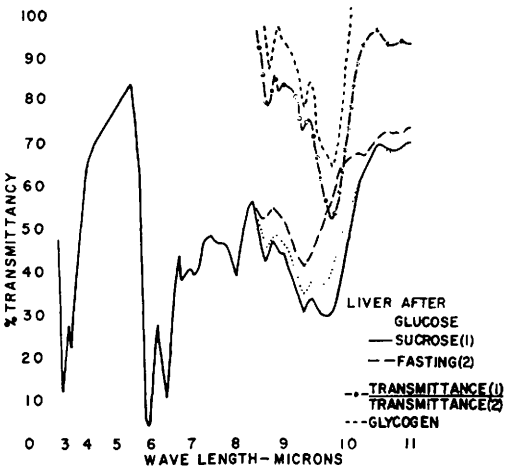

FIG. 2. Comparison of infrared spectra of liver of fasted and sugar fed litter mates and commercial glycogen.

The results of the assignment study strongly suggest that the characteristic strong band of normal liver (9.70 μ-9.60 μ) is produced by glycogen. The band at 9.23 μ is not caused by glycogen alone, but in part by vibrations of other substances. Almost complete disappearance of the 9.23 μ band after extraction of liver with hot trichloracetic acid indicates that it is produced in part by nucleic acids which have medium strong bands at that wavelength(6). The finding that the strongest band of glycogen in the 8.1-11 μ region is constantly observed at 9.70-9.60 μ in liver tissue is of practical importance. This "specific band" of the liver spectrum is distant enough from the "mixed band" at 9.23 μ that significant variations of its strength and location deserve careful consideration.

Semiquantitative estimation of liver glycogen. Ratios between optical densities of the glycogen bands at 9.70-9.60 μ and optical densities of the "protein bands" at 6.44 μ (designated as K values) have been used for semiquantitative estimation of the relative glycogen content of liver. With thickness of frozen sections kept at about 25 μ, it has been possible to obtain accurate measurements of bands and to calculate significant K values in dried rat liver sections with the exception

TABLE I. Quantitative Estimations in Liver Tissue.

Normal rats				Irradiated rats				
No. of rats		λ	K	No. of rats	Dose, r	Hr after exposure	λ	K
Adult				Adult				
Fasted	13	9.70	.279 ± .033*	Fasted 7	1800	48	9.63	.471 ± .019*
Non-fasted	27	9.60	.595 ± .019	Non-fasted 20	600–1800	48	9.60	.571 ± .027
Sucrose fed	9	9.65	.582 ± .021	" 8	19000	1½	9.60	.453 ± .015
Glucose fed	9	9.61	.449 ± .011					
Fetal				Fetal				
Fasted	5	9.67	.619 ± .065					
Non-fasted	24	9.70	1.002 ± .025	Non-fasted 20	250–400	24	9.71	.976 ± .062
Glucose fed	9	9.71	.853 ± .069					

λ = Wave length—μ; $K = \frac{\text{Optical density at } 9.70\text{–}9.60\ \mu}{\text{Optical density at } 6.44\ \mu}$

* Means ± S.E.

of those from fasted adult rats. In this instance, when K values were calculated for exactly 9.70 μ though no glycogen band was detectable, the values are not a measure of glycogen, but caused mainly by non-specific absorptions and energy losses from reflection and scattering. These K values of adult fasted rats are given only to show the order of magnitude and relative constancy of the unspecific factors involved in the semiquantitative glycogen estimations. No attempt at correction of K values for those factors was made since it is thought that such a correction would not improve the relative value of our estimations except in the case of very small glycogen contents. This spectroscopic method has been used for screening differences in liver glycogen of adult and fetal rats kept under various nutritional conditions as well as after total body x-ray irradiation. Significant results of these studies are summarized in Table I.

Liver glycogen in adult and fetal rats. The table demonstrates that K values of fetal liver are considerably higher than those of the adults, in the non-fasted state in excess of 68%, in the fasted state, over 120%. The spectroscopic differences are so great that they are many times apparent from simple inspection of the spectra. Maternal fasting of 24 hours duration, for example, distinctly reduces the K values in fetal liver, but enough glycogen has remained to produce a rather strong glycogen band. Fasting of same duration in the adult, on the other hand, reduces the liver glycogen to such low values, that the glycogen band disappears and K values calculated for 970 μ are mostly caused by the unspecific factors described previously. Difference of storage of liver glycogen in adult and fetal rats is also apparent from study of K values in sugar-fed animals. The relative amount of glycogen after sugar feeding is much higher in fetal than in adult liver.

Effect of ionizing irradiation on liver glycogen. As shown by the K values in Table I, x-ray irradiation produces both an "acute effect" and a "delayed effect" upon liver glycogen, dependent on the nutritional state of the animal and the time elapsed after the exposure. The "acute effect" which was observed in non-fasted rats 1½-3 hours after exposure to 19000 r is characterized by a moderate drop in liver glycogen. The "delayed effect" noted in fasted animals 48 hours after irradiation with 1800 r consists of a significant increase of liver glycogen. The "delayed effect" can be recognized even from comparison of infrared spectra of irradiated animal and fasted control (Fig. 3). As a result of these opposing effects, non-fasted animals do not show significant changes of liver glycogen up to 48 hours after exposure to moderately high doses of x-rays (adults, 600-1800 r; fetus, 250-400 r maternal irradiation) provided that diarrhea or loss of appetite do not influence food absorption (Table I).

Discussion. It is apparent from the data presented that qualitative and quantitative infrared spectroscopy of liver sections provide

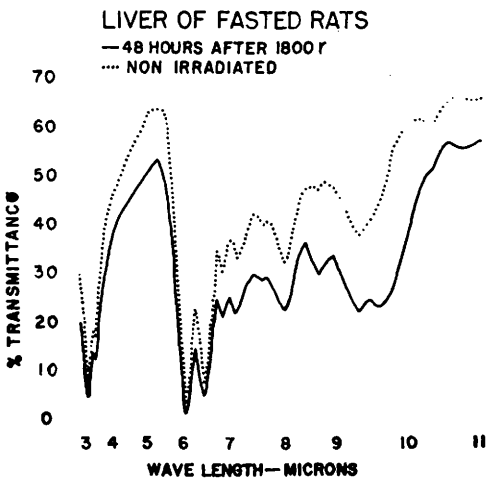

FIG. 3. Infrared absorption spectra of liver of non-irradiated and irradiated fasted rats.

an additional useful tool for study of liver glycogen. The variation of location of the "specific glycogen band" in adult and fetal liver is interesting when we consider that we did not find a single instance in which that band occurred exactly at 9.70 μ in normally fed adult animals, while it was regularly seen at that location in fetal liver and in commercial glycogen. Only in very few liver sections of adult animals fed with relatively high amounts of sucrose did we actually see that band close to 970 μ. This difference of location of the band exceeds by far the error of the method (± 0.01 μ) and statistical analysis of data from all groups of non-fasted rats proved it to be statistically significant ($P < 0.01$). Two possible explanations for this finding which are still under investigation are offered. 1. The differences in wavelength may be related to different branching of the glycogen molecule. This possibility would fit into the findings of Cori *et al.*(7) who observed differences of end group figures in adult and fetal glycogen. 2. The variation of wavelength may be due to different protein binding of glycogen, with "protein bonded glycogen" producing vibrations at wavelengths somewhat different from those of "free glycogen." The assumption that glycogen rich liver contains mainly free glycogen would be in agreement with the concept of Willstaetter(8) who thought that "in glycogen rich liver the protein content was insufficient to bind a larger part of the glycogen." This explanation is further suggested by recent studies in another species(9) which show that almost all fetal glycogen can be extracted with cold trichloracetic acid while adult glycogen is not so easily extractable in this way.

The high glycogen content of fetal liver previously described and its response to maternal fasting and feeding are in agreement with studies of previous investigators who considered fetal glycogen more "mobile" with a tendency to fall or rise rapidly with fluctuations of maternal blood sugar(10).

Our results on the effect of ionizing irradiation upon liver glycogen tend to clarify seemingly conflicting reports that small doses (500 r) raise liver glycogen in fasted animals (11) and extremely high doses (110000 r), which actually killed the animal in 3½ hours, lower it in non-fasted animals(12). Our findings confirm and amplify both reports. An "acute effect" setting in 1½-3 hours after irradiation, is associated with loss of glycogen and a "delayed effect" occurring 48 hours after exposure, with an increase. The "acute effect" can obviously be recognized only in the non-fasted and the "delayed effect" can be observed best in the fasted animal.

Summary. 1. Frozen and dried sections of adult and fetal liver were studied by infrared spectroscopy. 2. The infrared absorption band found at 9.70-9.60 μ is definitely assigned to glycogen. 3. Differences of location of this band in adult and fetal liver are discussed. 4. A spectroscopic method for semiquantitative estimation of glycogen in liver sections is described. 5. Relative glycogen content in adult and fetal liver under various nutritional conditions is reported. 6. Studies of liver glycogen after x-ray irradiation show the existence of an "acute" and "delayed" effect, the former decreasing, and the latter increasing liver glycogen.

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An Hemostatic Defect Associated with Dextran Infusion. (20798)

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(Introduced by Victor M. Sborov.)

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Dextran, a glucose polymer of high molecular weight, has been developed as a plasma expander(1). During the course of studies at Walter Reed Army Hospital on the metabolic effects of dextran, 2 patients developed a bleeding tendency. One patient, after receiving 6000 ml of 6% dextran over a 6-day period developed repeated epistaxis and bled from a duodenal ulcer. The second patient, after receiving 14000 ml of 6% dextran over a period of 14 days, bled for more than 30 minutes from an incised sebaceous cyst. The bleeding time and prothrombin time were prolonged in both cases. Detailed studies were initiated to determine whether the administration of dextran provoked an hemostatic defect.

Methods. Six lots of commercially available dextran from 4 manufacturers were tested. Studies were done on normal young adult subjects who received intravenously varying amounts of 6% dextran. Bleeding time was measured following a stab incision with a Bard-Parker No. 11 blade through the dependent ear lobe. Prothrombin activity was measured by the one-stage Quick method; the clotting time was done in siliconized glassware. Plasma coagulation factors V and VII (labile and stable factors), prothrombin consumption, platelet count, clot retraction, antithrombin titer, and plasma dextran levels were determined(2,3). The subjects in these studies were divided into 3 groups.

Results. The first group of 11 patients received 1000 to 1500 ml of dextran daily until the bleeding time was prolonged to greater than 10 minutes. The total amount of dextran required to prolong the bleeding time varied from 1500 ml given in a single infusion to 6500 ml given over a period of 5 days. Determinations were carried out at least 4 times before each study and at 3-hour intervals for 9 to 12 hours after infusion. The bleeding time exceeded 12 minutes in 4 subjects and 30 minutes in 7 subjects. The prolonged bleeding times were usually not present immediately after infusion, but were maximal 3 to 9 hours after completion of the last infusion. With one exception the bleeding time returned to normal in 24 hours. Concomittant with the increased bleeding time, progressive diminution of prothrombin activity was noted with each repeated infusion of dextran in all subjects. The maximum depression of activity after infusion varied from 75-50% of normal. This depression of prothrombin activity was partially corrected by the addition of factors V and VII. Fig. 1 depicts the typical changes observed.

The clotting time was never abnormal, even when the bleeding time exceeded 30 minutes. Platelet counts were not below 150000/cu mm